U.S. DEPARTMENT OF COMMERCE
National Technical Information Service
PB-299 404

Determination of Benzo(a)Pyrene and Other Polynuclear Aromatic Hydrocarbons in Airborne Particulate Material by Ultrasonic Extraction and Reverse Phase High Pressure Liquid Chromatography

(U.S.) Environmental Sciences Research Lab, Research Triangle Park, NC

28 Jul 78

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO.	2.	3.
EPA-600/J-78-128	JOURNAL ARTICLE	PB 299404

4. TITLE AND SUBTITLE
DETERMINATION OF BENZO(a)PYRENE AND OTHER POLYNUCLEAR AROMATIC HYDROCARBONS IN AIRBORNE PARTICULATE MATERIAL BY ULTRASONIC EXTRACTION AND REVERSE PHASE HIGH PRESSURE LIQUID CHROMATOGRAPHY

5. REPORT DATE

6. PERFORMING ORGANIZATION CODE

7. AUTHOR(S)
C. Golden and E. Sawicki

8. PERFORMING ORGANIZATION REPORT NO.

9. PERFORMING ORGANIZATION NAME AND ADDRESS
Environmental Sciences Research Laboratory
Office of Research and Development
U.S. Environmental Protection Agency
Research Triangle Park, NC 27711

10. PROGRAM ELEMENT NO.
1AD605 BE-08 (FY-76)

11. CONTRACT/GRANT NO.

12. SPONSORING AGENCY NAME AND ADDRESS
Environmental Sciences Research Laboratory--RTP, NC
Office of Research and Development
U.S. Environmental Protection Agency
Research Triangle Park, NC 27711

13. TYPE OF REPORT AND PERIOD COVERED

14. SPONSORING AGENCY CODE
EPA/600/09

15. SUPPLEMENTARY NOTES
REFERENCE: Analytical Letters, A11(12):1051-1062, 1978.

16. ABSTRACT

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17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
* Air pollution * Aromatic polycyclic hydrocarbons * Particles * Chemical analysis * Ultrasonic radiation * Chromatographic analysis		13B 07C 07D 20A

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES
RELEASE TO PUBLIC	UNCLASSIFIED	14
	20. SECURITY CLASS *(This page)*	22. PRICE
	UNCLASSIFIED	A02-A01

EPA Form 2220-1 (9-73)

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

EPA-600/J-78-128
JOURNAL ARTICLE

ANALYTICAL LETTERS, A11(12), 1051-1062 (1978)

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KEY WORDS: Air particulate material, polynuclear aromatic hydrocarbons, benzo(a)pyrene, reverse phase high pressure liquid chromatography, fluorometry, ultrasonic extraction, controlled pore glass powder.

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ABSTRACT

Ultrasonic extraction of airborne particulate material on Hi-Vol filters is described. Almost all of the polar compounds are removed during the extraction by adsorption on the surface of the shredded glass fibers and controlled pore glass powder (CPG). The non-polar polynuclear aromatic hydrocarbons (PAH) in the extract are separated at room temperature by high pressure liquid chromatography (HPLC) on reverse phase Vydac using acetonitrile:water (70:30 v/v) as the chromatographic solvent. There is baseline separation of benzo(a)pyrene (BaP), benzo(k)fluoranthene (BkFt) and of benzo(e)pyrene (BeP), benzo(b)fluoranthene and perylene, the latter three present in one band. Extracts of airborne particulates show the same peaks. BaP elutes in approximately 14 minutes. Precision and accuracy measurements indicate full recovery of PAH and good extraction reproducibility. The detection limit of BaP at F 290/389 is less than 10 pg. Total analysis time is approximately 1 1/2 hr, most of which is waiting time.

INTRODUCTION

Of the known carcinogens in airborne particulate material, the PAH have been the most studied, and special interest has always attached to the pentacyclic PAH, BaP[1]. Methods developed over the years for the extraction, separation, identification and quantitation of BaP have involved several steps, including Soxhlet extraction, various sublimation techniques[2], group separation by thin layer chromatography, HPLC, gas chromatography interfaced with the mass spectrometer and others[3-10]. It has been difficult to determine in one step a single compound such as BaP present in extracts of air particulates containing hundreds of compounds. Improved microparticle reverse phase stationary phases for HPLC give sharp separations which appear to bring this goal nearer[5,6]. The strong fluorescing and absorbing characteristics of PAH provide the requisite sensitivity and specificity for identification and the determination of the purity of the chromatographic peaks[2,8].

The present procedure consists of an improved ultrasonic extraction of airborne particulates collected on glass fiber filters, followed by a reverse phase HPLC separation, and the identification and quantitation of BaP and other PAH by fluorescence detection at low picogram levels.

EXPERIMENTAL

Instrumentation. Shredding of the Hi-Vol filters containing the air particles was done by a Branson W 185 Sonifier Cell Disruptor (20 KHz power ultrasonic generator with a 1.27 cm (1/2 inch) horn disruptor) in a sonabox from Heat Ultrasonics Inc. (13). The shredding vessel was a water-jacketed hand-blown glass funnel 3.8 cm I.D. x 10 cm tall with a medium sintered glass filter and a 4 mm Teflon stopcock in a 12.5 cm (5 inch) stem as shown in Figure 1. It is used with a Fisher Filtrator and 350 ml hand-blown straight-sided glass stoppered flask to filter the extracts. Controlled pore glass powder (CPG) Code 7930 200/325 mesh 40 Å was developed by the Corning

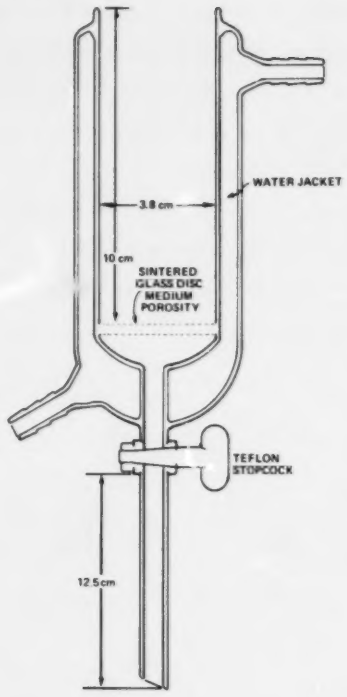


FIG. 1.

Water-Jacketed Glass Funnel for Ultrasonic Extraction and Filtering of Extracts of Airborne Particulates. Overall length 28.5 cm.

Biological Products group and is sold by the Pierce Chemical Company. Extracts were concentrated with the use of an Ace adapter to collect the evaporated solvent. Final evaporation to dryness was done in the Meyer N-Evap Analytical Evaporator (model No. 111, from Organomation Associates, Inc.) under a stream of nitrogen gas. The reverse phase separation was done with a Spectra-Physics 3500 Liquid Chromatograph with a Schoeffel FS 970 Fluorometric Detector. The recorder was a Hewlett-Packard strip chart with ball-disk integrator. The column was stainless steel, 3.2 mm x 250 mm

prepacked with Vydac 201 TP 10 micron RP. Used CPG was cleaned by heating in a muffle furnace. Three valves connected in series were installed on the panel of the oven of the chromatograph by Valco-Valve of Houston, Texas. They provide a 10 or 20 µl, a 5 ml loop injector and a column bypass. A schematic diagram is shown in Figure 2.

Cyclohexane, acetonitrile and methanol from Burdick and Jackson were distilled in glass. Pure PAH standards were obtained from Dr. E. J. Eisenbraun of Oklahoma State University under Grant No. 805419. Water was deionized by passing tap water through a filter, activated charcoal and two ion exchange columns.

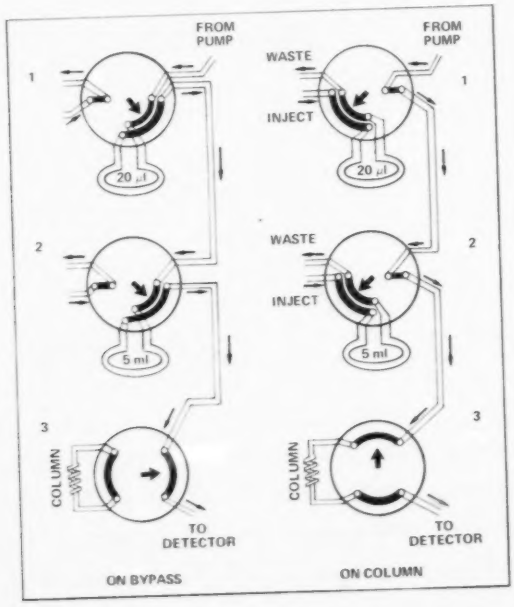


FIG. 2.

Schematic of Two Six-port Loop Injectors Valves and One Four-port Column Bypass Switching Valve.

Extraction A sixteen square centimeter section of glass fiber filter on which air particles were collected was cut into strips roughly 0.25 x 1 cm. The strips were placed in the sonifying vessel, along with 1 g CPG and 60 ml of 35% methylene chloride in cyclohexane. Sonification was carried on for five minutes at 60 Watts with the end of the probe approximately 1.5 cm above the bottom of the vessel. The extract was filtered into the flask in the Fisher Filtrator, and the shredded sample was rinsed. Sixty ml more of the solvent was added and the extraction repeated for four minutes. The filtrate and rinsings were added to the first filtrate. The extract was concentrated to a volume of approximately 15 ml with the aid of the distilling adapter and with glass beads to prevent bumping, transferred to a 30 ml glass-stoppered reagent bottle and evaporated to dryness in the N-Evap at 40-50°C under a stream of nitrogen gas. The contents of the bottle were dissolved in 1 ml or more of methanol, depending on the degree of pollution of the sample. Extracts were stable for several days at room temperature in the dark, longer if refrigerated.

The CPG was prepared by washing with 10% hydrochloric acid and rinsing voluminously with water. The fine particles which did not settle out were discarded. The CPG was air-dried for several days. Approximately 95% of the used CPG was recovered by sifting first through a 180 mm then a 106 mm sieve: it was then heated to 500°C for up to 2 hrs in the muffle furnace to burn off the adsorbed carbon and organic material. After cooling, it was soaked in water overnight, washed and air-dried.

Chromatographic Separation. Twenty microliters of the extract was separated on the Vydac reverse phase column with acetonitrile:water (70:30 v/v) as solvent at a flow rate of 1 ml/minute. Detection was at F 290/389 on the Schoeffel Fluorometer at range 0.20. Temperature was controlled at 25°C and the pressure drop was 3000 psi.

Chromatograms of mixed standards (C), air particle extract (B), and extract to which BaP and BkFt standards were added (A), are shown in Figure 3. The pentacyclic PAH standards elute in three peaks[4-6], and the extract shows three pentacyclic peaks identical in retention time to the standards. The addition of BaP and BkFt standards does not modify the retention times

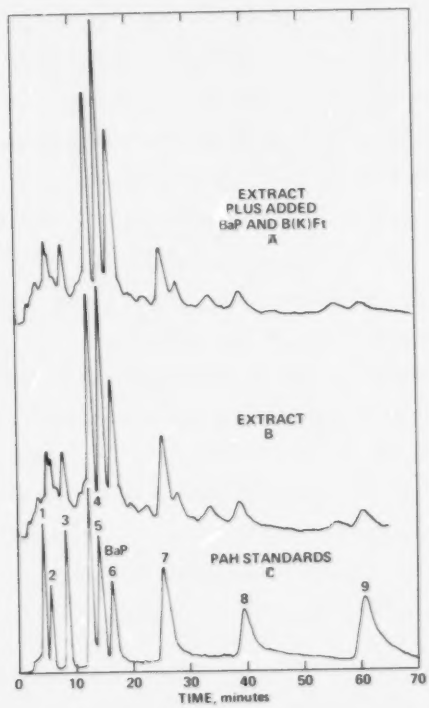


FIG. 3

Chromatograms of Mixed PAH Standards, Extract of Airborne Particulates and Extract to Which BaP and BkFt Standards Have Been Added.
(1) Anthracene, 78 ng (2) Pyrene 0.4 ng (3) Chrysene 14.7 ng (4) BeP 8 ng, Perylene 12 ng, BbFt 0.15 ng (5) BkFt 0.15 ng (6) BaP 0.4 ng (7) Benzo(ghi)-perylene 1.2 ng, (8) Anthanthrene 0.95 ng (9) Coronene 2.74 ng.
Vydac 201 TP 10 micron RP in a 3.2 mm x 250 mm column; solvent acetonitrile: water (70:30 v/v); flow rate 1 ml/min.; 25°C; 3000 psi; F 290/389, range 0.20.

of the corresponding extract peaks, which is strong evidence for their identity. The extract also has peaks with the same retention times as benzo(ghi)perylene, anthanthrene, and coronene in addition to several others.

Chromatograms of extracts D, E and F from different sites (Figure 4) show peaks in the same places; they vary only in peak heights, as did all the air particle samples studied. Many of these peaks could be characterized and quantitated with appropriate sensitive instrumentation.

The BaP elutes in approximately 13 to 15 minutes and after it is off the column, the more slowly eluting compounds can be flushed off in 9 min-

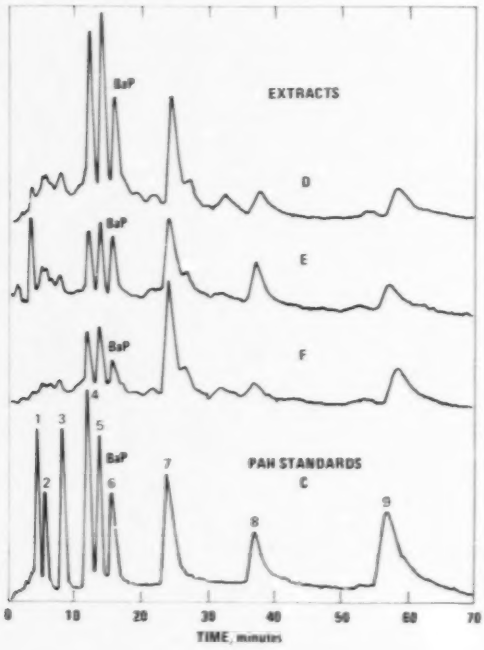
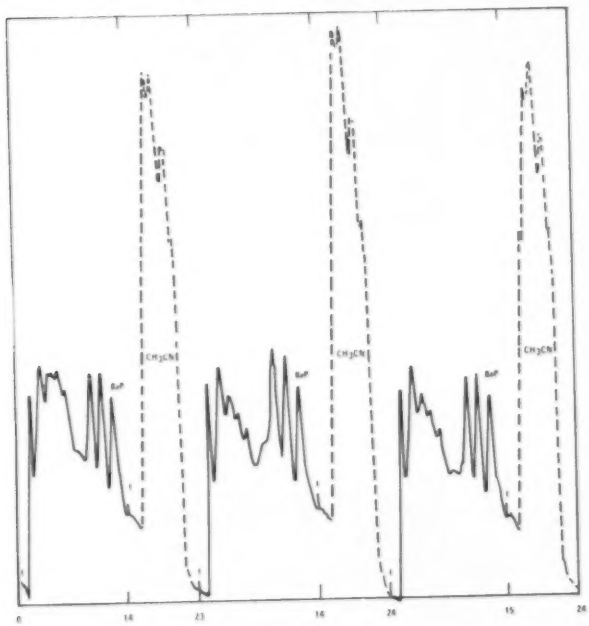


FIG. 4

Chromatograms of Mixed PAH Standards and Extracts of Airborne Particulates from Three Different Sites.
Standards and chromatographic conditions the same as in Figure 3.

utes by a slug of acetonitrile through the 5 ml loop injector. Another extract sample can be injected immediately as shown in Figure 5. Other more polar compounds remain on the column without apparently interfering with the separation. They are stripped off occasionally by pumping acetonitrile through the column; methylene chloride may also be used.

Maximization of Extraction Parameters. The amount of CPG was maximized by extracting replicate composited homogeneous air particulate Hi-Vol samples



TIME, Minutes

FIG. 5

Chromatograms of an Air Particle Extract.
After the BaP is eluted, an injection of acetonitrile through the 5 ml loop injector strips off most of the more polar material and another sample injection may be made immediately. Sample injections may be made approximately every 23 min. Chromatographic conditions are the same as in Figure 3.

and blank glass fiber filter samples on which BaP standard was adsorbed, with amounts of CPG varying from 0.0 to 4.0 g.

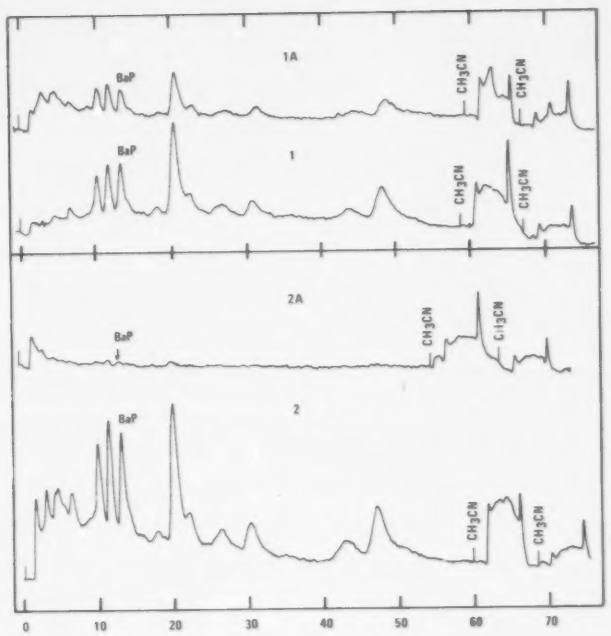
The peak areas of the total particulate aromatic hydrocarbons (TpAH) in the extract were obtained on the column bypass at a flow rate of 0.48 ml/minute and range 1.0 on the Schoeffel Fluorometer. Measurements of BaP in the extract were made after chromatographing the extract on the column at 1 ml/min, range 0.20. The relative standard deviation of the recovery of BaP by this procedure was ± 2.55%. One g of CPG gave maximum extraction of BaP with minimal extraction of 1 to 4 ring PAH and large, more polar compounds. Standard BaP was not lost even when as much as 4 g of CPG was used.

Sonifying time was determined similarly using 1 g of CPG and varying the time from 6 to 30 minutes. Maximum recovery of BaP was reached in 8 minutes and the level of TpAH was still rising at 30 minutes. Nine minutes was taken as a safe and effective sonifying time. However, sonifying time can vary with the thickness and stiffness of the glass fiber filter. It is recommended that sonifying time be maximized for each kind of filter used.

DISCUSSION

In the previous work on ultrasonic extraction[3] with cyclohexane as solvent, approximately 15% more TpAH was extracted than by Soxhlet refluxing. However, the recovery of added BaP standard does not necessarily mean that all the BaP in the air particle sample is removed, and in fact it is not. More BaP and other PAH are extracted when the shredded sample left in the sonifying vessel after the cyclohexane extraction is reextracted with solvent of incrementally increasing polarity. It is necessary to use 35% methylene chloride in cyclohexane to remove all except traces of the pentacyclic PAH, as shown in Figure 6.

The smaller the inner diameter of the ultrasonic vessel and the lower the ultrasonic probe is positioned, the shorter the sonifying time necessary



TIME, Minutes

FIG. 6

Chromatograms of Extracts of Air Particulate Material.
(1) Extracted with cyclohexane. (1A) Sample 1 reextracted with pentant:methylene chloride (66:34 v/v). Indicates much PAH was not removed by the first extraction with cyclohexane. (2) Extracted with cyclohexane:methylene chloride (65:35 v/v). (2A) Sample 2 reextracted with the pentane:methylene chloride solvent. Only traces of the pentacyclic PAH and benzo(ghi)perylene were obtained, indicating they were removed by the cyclohexane methylene chloride solvent. The slugs of acetonitrile injected at the end of the runs indicate that very little material was more polar than the PAH that remained on the column.

for maximum extraction of PAH. But the closer the end of the probe to the bottom of the vessel, the greater is the possibility of the sintered glass disc cracking eventually under the impingement of the ultrasonic waves.

Sintered glass discs vary greatly in hardness, and the hard discs last longer than the soft ones. An extra disc laid on the bottom of the vessel

presents no technical problem and appears to absorb the impact of the waves, thus protecting the filter.

A two-piece vessel is in work, but the difficulty is to water-jacket the top over a sufficiently large area to maintain the temperature of the contents.

Many adsorbents were screened but Corning CPG was the only one found to adsorb polar compounds from an extract in a non-polar solvent (11) in a test-tube without also removing the BaP. CPG enhances the shredding action of the ultrasound and, along with the shredded glass fibers, adsorbs the finely divided carbon and polar compounds present. As stronger solvents are used, the carbon still is adsorbed and larger amounts of more polar material are extracted. With appropriate solvents there is the possibility that all the remaining compounds in the finely divided air particulates in the sonifying vessel can be extracted, separated chromatographically, identified and quantitated. This study is now being initiated.

The simple calculations are the same as for TpAH[3], except that the peak area/m^3 of air of each PAH can be converted to weight by referrence to a standard curve. The area of the filter sample, the area of the whole filter and the volume of the loop injector are constants. The volume of the extract may vary, and the only other variables are the volume of air sampled and the peak area of the compound analyzed.

CONCLUSION

The greater efficiency and speed of the ultrasonic extraction over the traditional Soxhlet extraction, the small amount of polar material in the extracts which makes possible a cleaner separation of PAH, the efficiency of the Vydac separation, the extreme sensitivity of the fluorescence measurement, the absence of pretreatment of the Hi-Vol filters, and the simplicity of the calculations, make it appear that this procedure should be economically feasible for routine analysis for various PAH, especially BaP,

found in air particulate extracts. In addition it offers very interesting possibilities for determining other compounds in air particulates, many of which are carcinogenic[12].

ACKNOWLEDGEMENTS

We thank the Corning Biological Products Group for the controlled pore glass powder. We are also grateful to Mr. James Meeker for many helpful discussions, and to Ms. Sally Hubbard for assistance in the assembling of the switch valves.

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Received July 21, 1978
Accepted July 28, 1978